OFFICE OF NAVAL RESEARCH

CONTRACT N00014-94-C-0149

TECHNICAL REPORT 97-11

# FREEZING BABOON RED BLOOD CELLS IN THE ORIGINAL 800 ML POLYVINYLCHLORIDE PLASTIC BAG WITH 40% W/V GLYCEROL AND STORAGE AT -80 C AND DEGLYCEROLIZATION USING THE IBM COBE BLOOD PROCESSOR 2991

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12 AUGUST 1997

## I. INTRODUCTION

A method is described for the preparation and storage of baboon red blood cells, frozen, in the original polyvinylchloride (PVC) plastic collection bag. Up to four hundred fifty (450 + 45 ml) ml of blood is collected in a quadruple plastic bag system with a special adaptor port on the tubing connecting the primary 800 ml PVC plastic bag to the three transfer packs. The red cells are concentrated by centrifugation of the primary collection bag and all of the plasma is expressed into one of the integrally attached transfer packs. The red cell concentrate in the 800 ml primary collection bag with a hematocrit of 75 $\pm$ 5 V% is stored with an empty integrally attached transfer pack. Glycerol solution is added through the collection line of the primary 800 ml collection bag. Three aliquots of glycerol solution are introduced with short equilibration periods between each addition. The red cells are then concentrated by centrifugation, the supernatant glycerol is transferred into the empty integrally attached transfer pack and discarded. The glycerolized packed red cells are then frozen. The procedure uses a high concentration of glycerol (40% W/V) and mechanical refrigeration at -80 C for the freezing and storage of the product.

To freeze red cells in the 800 ml primary PVC plastic bag, it is essential that only 450 ml of blood be collected in the primary bag containing 63 ml of CPDA-1. The method used to prepare the red cell concentrate from blood must ensure that the hematocrit of the concentrated red cells is 75 $\pm$ 5 V%. Blood collected into the 800 ml quadruple plastic bag system can be stored at 22 C for up to 8 hours. The blood is centrifuged at 1615 X g for 4 minutes in a refrigerated centrifuge maintained at 22 C and all the visible plasma removed into one of the three transfer packs (Table 1). It is essential that all the visible plasma be removed to prepare a red cell concentrate with a hematocrit of 75 $\pm$ 5 V%. Both the volume of blood collected and the hematocrit of the red cell concentrate ensure that the 800 ml volume primary PVC plastic bag is adequate to ensure proper mixing of the red cells and glycerol solution.

Red cell concentrates are stored at 4 C for 3 to 6 days (inclusive) before they are glycerolized and frozen as described below.

## II. BLOOD COLLECTION AND COMPONENT PREPARATION

Anesthetize the baboon according to the NBRL SOP for baboon anesthetization. Established phlebotomy guidelines should be followed. Be sure to adjust the balance or vacuum assist device so that a maximum of 450 ml of blood is collected into the 800 ml primary bag of the quadruple plastic bag collection system (Cutter #746-74; Fenwal #4R1243). Weigh the baboon. According to approved NBRL protocols for baboon blood collection, the maximum volume of blood which can be drawn from an individual baboon is approximately 10 ml/kg.

The quadruple bag collection system has a primary bag with a volume of 800 ml. Each primary collection bag contains 63 ml of citrate-phosphate-dextrose-adenine (CPDA-1) anticoagulant. After collection of blood into the primary collection bag, the unit is processed as follows:

Within 8 hours of collection the primary bag is inverted and the bag is then folded back about 2 inches from the base and secured with tape. The folded bag is placed upright in a refrigerated centrifuge. The blood is centrifuged at 1615 X g for 4 minutes (no brake) to prepare a red cell concentrate with a hematocrit value of 75 $\pm$ 5 V% (Table 1). All the plasma is removed to one of the integrally attached transfer packs and frozen in a special cardboard box at -80 C. The red cells are stored in the 800 ml primary bag, along with the adaptor port on the tubing connecting the primary bag and transfer pack at 4 C for 3 to 6 days (inclusive) and then frozen.

## III. CONSUMABLES

1. 800 ml CPDA-1 quadruple blood pack (Cutter #746-74; Fenwal #4R1243);

2. Hand sealer clips (Fenwal #4R4418);

## IV. GLYCEROLIZATION

### a. Starting Components

Red cell concentrates (hematocrit values of 75-80%) in the 800 ml PVC plastic collection bag with an adaptor port on the plastic tubing connecting the 800 ml plastic bag to the empty transfer pack are stored at 4 C for 3 to 6 days (inclusive) prior to glycerolization.

**b. Materials**

**CONSUMABLES**

1. Glycerol:

   A. Glycerolyte 57 (6.2 M glycerol, 500 ml bottle). Each 100 ml contains 57 g glycerin, 1.6 g sodium lactate, and 30 mg potassium chloride, buffered with 51.7 mg monobasic sodium phosphate (monohydrate) and 124.2 mg dibasic sodium phosphate (dried), pH 6.8 (Fenwal #4A7833)

   B. 6.2 M Glycerolizing Solution (500 ml bottle). Each 100 ml contains 57.1 g glycerin, 1.6 g sodium lactate, and 0.03 g potassium chloride, buffered with 43 mg monobasic sodium phosphate and 220 mg dibasic sodium phosphate, pH 7.0 (Cytosol PN-5500)

2. Plasma transfer set with two couplers (Fenwal #4C2243);

3. Sterile filtered airway needle (B-D 5200);

4. Labels for the primary collection bag and for the cardboard storage box

5. Heat-sealable plastic bags (3), 8" X 12" (Kapak/Scotchpak 404);

6. Corrugated cardboard storage box. (Dimensions: 7" X 5.25" X 2" outside);

7. Alcohol swab (70%) (B-D 6894);

**c. Temperature Monitoring**

At the time of glycerolization, the red cells, glycerol solution and room temperature should be within a temperature range of 20 C (68 F) to 26 C (80 F). The temperature of a bottle of glycerol located in the storage area should be monitored by inserting a calibrated thermometer into the full bottle of glycerol. If the glycerol is below 20 C, the glycerol can be warmed to a temperature of 20-30 C by incubation at 37 C for the appropriate time to achieve the desired temperature. The red cells can be warmed by either of the following manipulations:

A. Remove the liquid red cell concentrate from refrigerated storage and store at room

temperature (20 C to 30 C) for a period of about 2 hours.

B. Remove the liquid red cell concentrate from refrigerated storage and place it in sealed, double plastic bags for protection against wetting. Immerse the double-bagged units in a 37 C water bath for 20 minutes.

***NOTE:*** *Each plastic overwrap bag must be flattened to remove all the air prior to sealing. If this is not done properly, the units will float on the surface of the water bath during incubation, and the desired temperature will not be achieved.*

1. The primary collection bag containing the red cell concentrate, the integrally attached transfer pack and adaptor port are placed in a plastic bag, and the bag is heat sealed.

2. The sealed plastic bag is placed inside a second plastic bag; this bag also is heat-sealed. Lead weights are placed on the double-overwrapped red cell unit to keep it submerged in the water bath.

3. Turn on the power switch of the water bath located at the end of the water bath. Allow the water to warm to 37 C (approximately 1 hour). Switch on the circulating pump in the water bath a few minutes prior to use to ensure a uniform temperature of 37 C throughout the bath. Temperature is measured with a thermometer that has been verified against a National Bureau of Standards (NBS) certified thermometer.

4. Place the overwrapped unit(s) in the water bath, and keep submerged by placing lead weigths on top of the unit.

5. Incubate the unit in the 37 C water bath for 20 minutes; at the end of 20 minutes the temperature of the red cells should be approximately 20 C.

6. Remove the bag from the water bath; wrap the unit loosely in a clean, dry disposable towel, dry the surface of the overwrap, and remove the plastic overwraps from the primary bag, assuring that the inner bag and unit are not contaminated with any water from the water bath.

7. The red cells are now ready for glycerolization.

8. Alternatively, the liquid red cell concentrate can

be warmed using the Thermogenesis or Instacool plasma thawer. Remove the red blood cell concentrate from refrigerated storage and place it in the pouch of a Thermogenesis or Instacool plasma thawer maintained at 40 C. Immerse the non-overwrapped unit in the plasma thawer for 2-4 minutes.

**d. Glycerolization**

1. Weigh the unit and record the weight on the glycerolization worksheet. The gross weight includes the red cell concentrate and the primary collection bag alone. The gross weight must not exceed 372 grams. The net weight is the weight of the red cell concentrate minus the weight of the primary collection bag. The net weight must not exceed 330 grams. The net weight for the 800 ml primary bag is 42 grams.

2. Check the hand sealer clip on the tubing between the collection bag and the transfer pack to be sure it is still in place (not crimped).

3. Remove the plasma transfer set (Fenwal 4C2243) from its box and close the roller clamp.

4. Sterilely dock one of the coupler ends of the plasma transfer set (Fenwal 4C2243) to the collection line (Figure 1). Squeeze weld.

5. Mount the primary collection bag containing the red cells on the shaker platform.

6. Remove the metal pull tab from the top of the glycerol bottle, swab the rubber stopper with an alcohol swab (70%), and then aseptically insert the remaining coupler of the plasma transfer set into the outlet port of the glycerol bottle stopper.

7. Insert a filtered airway needle into the vent port of the glycerol bottle stopper. As the bottle vents, invert the glycerol bottle and install it on the support stand hook provided on the shaker so that the rubber stopper on the bottle of glycerol is held 18 inches (45 cm) above the level of the primary bag on the shaker.

8. Place the 800 ml primary collection bag containing the red cells on the shaker platform; allow the attached transfer pack to hang over the shaker platform.

9. Using Table 2 and the previously recorded gross or net weight, determine the volume of glycerol solution to be added to the red cells during each of the three glycerol

addition steps. Using the factory graduations as a guide, mark the volume of glycerol to be added for each of the three steps.

10. Switch the modified Eberbach shaker on low speed (180 oscillations/minute). Check to be sure the hand sealer clip is in place on the line between the primary bag and the empty transfer pack to prevent the glycerol from flowing into the transfer pack.

11. Open the roller clamp of the plasma transfer set (Fenwal 4C2243) and add the first volume of glycerol from the solution bottle directly into the primary bag containing the red cells.

12. Close the roller clamp, turn off the shaker and equilibrate the red cells for 5 minutes.

13. Switch the modified Eberbach shaker on low speed.

14. Open the roller clamp and add the second volume of glycerol from the solution bottle directly into the primary bag containing the red cells.

15. Close the roller clamp, turn off the shaker and equilibrate the red cells for 2 minutes.

16. Remove unit from shaker.

17. Open the roller clamp and using continuous, vigorous manual agitation, allow the third volume of glycerol (final volume) to enter directly into the primary bag.

*NOTE: During the addition of the third volume of glycerol, only 2 units should be processed at a time to ensure proper vigorous manual mixing, one unit held in each hand.*

18. Close the roller clamp and heat seal the tubing between the empty bottle of glycerol and the draw line, ensuring that the transfer pack remains integrally attached to the primary collection bag.

19. Discard the empty glycerol bottle and connector tubing.

20. Spin the glycerolized red cells at 1248 X g in a 22 C refrigerated centrifuge (Sorvall or Beckman) for 10 minutes (Table 1).

*NOTE: <u>The brake on the centrifuge should be set at zero. This brake setting will minimize red cell</u>*

*mixing which occurs as the rotor slows down from maximum to zero.*

21. Carefully remove the unit from the centrifuge and place it on a plasma extractor.

22. Remove the hand sealer clip from the tubing between the collection bag and transfer pack.

23. Express all visible supernatant glycerol from the primary bag into the transfer pack to achieve a hematocrit of 60 $\pm$ 5 V% and resuspend and mix thoroughly by manual agitation. The glycerolized red blood cell concentrate must be resuspended completely before freezing to prevent hemolysis.

24. Seal the tubing 6 inches from the primary collection bag and detach the transfer pack containing the supernatant glycerol, and discard.

25. It is important that the label contain information describing that the bag contains **baboon** red blood cells.

26. Weigh the unit just prior to freezing and record the gross weight of the glycerolized red cells.

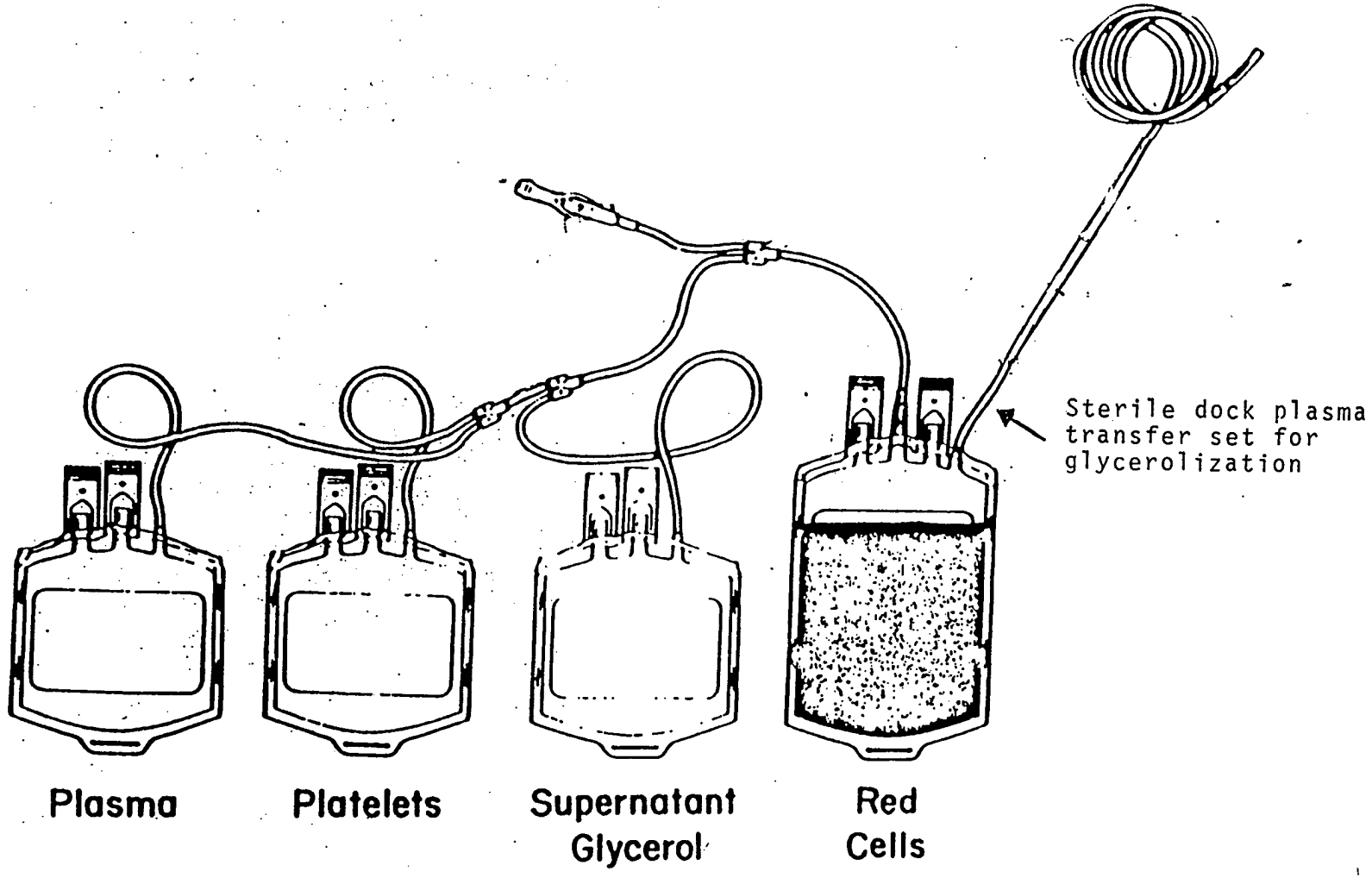
27. Fold over the top portion of the primary bag (approx. 2 inches) and then place the unit into a plastic bag overwrap (8" X 12") and seal across the top using an impulse sealer so that there is as little trapped air as possible. The plastic bag will not break during freezing and the sealer will provide an air-tight and leakproof seal to ensure protection of the unit at the time of thawing. Make sure that the ports and tubing segments are folded beneath the unit so that they are protected from breakage when frozen.

28. Place the plastic bag containing the glycerolized red cells into the cardboard box and close the box Place the cardboard box in a -80 C freezer designated for freezing and storage of baboon red blood cells. Each unit should be frozen at the bottom of the -80 C freezer during the initial 24-hour period to ensure proper freezing. To avoid improper freezing, the units should not be stacked on each other. After the initial 24-hour period of freezing at the bottom of the -80 C freezer, the frozen units can be stored in other -80 C freezers, and stacked.

***NOTE:*** *No more than 4 hours should be allowed to lapse between the time the red cells are removed from the 4 C refrigerator and the time they are placed in the -80 C freezer. The final concentration of glycerol*

*is approximately 40% W/V and the hematocrit of the glycerolized unit is approximately 60 ± 5 V%.*

FIGURE 1




800 ml PRIMARY PVC PLASTIC BAG COLLECTION SYSTEM

## V. THAWING

### a. INTRODUCTION

A unit of glycerolized frozen red cells is thawed rapidly by immersion in a heated water bath maintained at 42 C for approximately 40 minutes or a Thermogenesis Plasma Thawer maintained at 40 + 2 C for approximately 35 minutes; the thawed red cells are warmed to a temperature of approximately 32 C). The temperature of the water bath or the Thermogenesis is measured with a thermometer that has been verified against a National Bureau of Standards (NBS) certified thermometer. If the frozen unit is to be thawed in a Thermogenesis plasma thawer, the plastic overwrap on the frozen red blood cells can be removed prior to placing the unit in the Thermogenesis to hasten thawing. However, water baths are highly susceptible to microbial contamination and if the unit is to be thawed in a water bath the overwrap must remain on the frozen unit prior to placing it in the water bath. It is imperative when thawing frozen blood products that local Standard Operating Procedures established for the control of contamination of water baths be rigorously adhered to.

### b. PROCEDURE

1. Latex gloves must be worn throughout this procedure.

2. a. Waterbath: Turn on the power switch of the water bath located at the end of the water bath. Allow the water to warm to 42 C (approximately 1 hour). Switch on the circulating pump in the water bath used to thaw the frozen red cells. Allow the pump to run for 1-2 minutes, then check the water temperature to ensure that it has stabilized at 42 C. Record the temperature of the water bath on the deglycerolization worksheet.

   b. Thermogenesis Plasma Thawer: Turn on the power switch and allow the thawer to warm to 40 $\pm$ 2 C.

3. Using freezer gloves, remove the cardboard storage box containing the red cells from the freezer.

4. Open the cardboard freezing box and remove the unit of frozen red cells.

5. a. Waterbath: Thaw the unit still in its plastic overwrap by immersing it in the water bath. Place

lead weights on top of the units so that the units remain submerged during the thawing procedure.

b. Thermogenesis: Remove the overwrap covering the frozen unit. Place the unit into one of the pockets of the plasma thawer.

*Using both methods, the unit is thawed to a temperature of approximately 32 C.*

6. Remove the unit from the water bath/Thermogenesis and check the temperature of the unit. To estimate the temperature, place the overwrapped unit onto the back of your hand or scan the surface of the back using an infra red scanner. Manually massage the unit to detect the presence of ice. If the unit feels cold to the touch or ice is palpable, replace the unit back into the water bath or plasma thawer. As described above, re-check the temperature every 5 minutes until the desired temperature is achieved.

7. a. Waterbath: Remove the unit from the water bath and dry off the overwrap. Tear open the overwrap and discard it.
b. Thermogenesis: Remove the unit from the plasma thawer.

For both thawing methods, wrap the thawed unit loosely in a disposable white towel. Check the bag for any breaks by gently compressing the unit in the towel, wiping the entire bag surface with the towel and then inspecting the towel for blood stains. The presence of blood stains on the towel is evidence of bag breakage, and the unit must be considered contaminated.

8. The thawed glycerolized red cells are now ready for deglycerolization.

## VI. DEGLYCEROLIZATION

### a. PRINCIPLE

The IBM-Cobe Blood Cell Processor Model 2991-1 is used to deglycerolize baboon red blood cells frozen with 40% W/V glycerol in the original 800 ml PVC plastic collection bag. This protocol describes the procedure to deglycerolize frozen red blood cells with normal or reduced volumes using a serial centrifugation, "batch wash", method using up to 50 ml of 12% sodium chloride (NaCl) solution, 1 liter of 1.6% NaCl solution, and 1 liter of 0.9% NaCl-0.2% dextrose.

### b. SPECIMEN REQUIREMENTS

Thawed glycerolized red blood cells frozen in the original 800 ml collection bag with 40% W/V glycerol.

### c. EQUIPMENT

1. IBM Cobe Blood Processor, Model 2991-1
2. Integral tubing sealer (Sebra Model 1100)
3. Water bath, 42 C (Blue-M, Fisher Catalog #15-4530) with circulating pump (Fisher #13-874-80) or Plasma Thawer (Thermogenesis, set at 40 $\pm$ 2C)
4. Adjustable slide clamps (2)
5. Hemostats
6. Pair of parallel jaw pliers

### d. MATERIALS AND REAGENTS

1. Cell wash set (Cobe #912647819)
2. 12% sodium chloride solution in 150 ml plastic container (Each 100 ml contains: 12 g NaCl, approximate pH 6.0, Fenwal #4B7874)
   NOTE: 50 ml or less is required for each unit processed.
3. 1.6% sodium chloride solution in 1 liter plastic container (Each 100 ml contains: 1.6 g NaCL, approximate pH 5.5, Fenwal #4B7870)
4. 0.9% sodium chloride-0.2% dextrose solution in 1 liter plastic container (Each 100 ml contains: 900 mg NaCl USP, 200 mg dextrose, approximate pH 5.0, Fenwal #4B7877)
5. 600 ml transfer pack with coupler (2) (Fenwal #4R2021)
6. Spike to spike transfer set (Fenwal #4C2243)

**e. PROCEDURE**

1. Turn the cell washer power switch on, allow machine to warm up, prime the pump as described by the manufacturer, and set the control panel settings.

2. Install the cell wash set as described by the manufacturer.

3. Insert one spike of the spike to spike transfer set into the 12% NaCl solution and the other into the plastic bag containing the thawed red blood cells.

4. Determine the amount of 12% NaCl solution to add based on the enclosed nomogram (Table 3). This value can also be calculated using the following formula:

a. Weigh the unit; determine net weight
b. Measure the hematocrit of the unit using the microhematocrit method
c. Determine the density using the following formula:

$$1.1 + \{(\text{hematocrit} - 20)/1000\}$$

d. Divide the net weight of the unit by the density to achieve the volume of blood in the unit
e. Multiply the volume by 1 - hematocrit (as a decimal) to determine the supernatant volume
f. Divide the supernatant volume by 3 to determine the volume of 12% NaCl to add to the thawed glycerolized red blood cells.

5. Place a reusable adjustable clamp onto the spike to spike transfer set. Open the clamp, and add the 12% NaCl solution to the red blood cells at a flow rate of 10-15 ml/minute. Mix the bag as the 12% NaCl enters with constant manual agitation. Close the clamp.

6. Remove the spike from the bag of 12% NaCl solution and put it into the 1.6% NaCl solution. Open the clamp, and add 100 ml of the 1.6% NaCl solution to the red blood cells at a flow rate of 10-15 ml/minute. Mix the bag as the 1.6% NaCl enters with constant manual agitation. Close the clamp.

7. Allow the red cells to equilibrate for 2 minutes. Heat the seal the tubing on the plasma transfer set and remove the spike from the 1.6% NaCl solution.

8. Open the clamp and add 150 ml of the 1.6% NaCl solution to the red blood cells at a flow rate of 20-25 ml/minute. Mix the bag as the 1.6% NaCl enters with constant manual agitation. Close the clamp.

9. Allow the red cells to equilibrate for 2 minutes. Heat-seal the tubing on the plasma transfer set and remove the spike from the 1.6% NaCl.

10. Insert the coupler of the cell wash set (red color-coded tubing) into the remaining port of the 800 ml primary collection bag containing the thawed-diluted red cells. Insert the green tubing into the 1.6% NaCl solution and the yellow tubing into the 0.9% NaCl-0.2 gm% glucose solution. Invert and hang the diluted red cells on the hook provided on the left side of the cell washer. Clamp off the blue-coded tubing on the cell washer set as it will not be required.

11. Press the button labeled "Blood In" and allow the red cells to enter the wash bowl.

12. When all the thawed-diluted red cells have entered the bowl, press the button labeled "Air Out".

*NOTE: The "Blood In" button must be on.*

13. Allow the blood cell processor to express all of the trapped air out of the bowl and back into the empty primary bag. When complete, press the "Blood In" button first, and then the "Stop Reset" button.

14. Press the button labeled "Start Spin".

a. Spin down the thawed-diluted red cells for 2.5 minutes.

*NOTE: The centrifuge should be spinning at 3000 rpm.*

b. When centrifugation is complete, select the "Supernatant Out" mode. The solenoid which occludes the tubing (color-coded purple) will open and all the visible waste in the bowl will be transferred into the waste bag.

c. When the red cell interface reaches the electric eye, press "agitate/wash".

d. Set the machine to valve 2. Open the reusable adjustable clamp on the 1.6% NaCl solution and allow the first of 2 volumes of 1.6% NaCl solution to enter the agitating bowl. The volume of 1.6% NaCl solution to enter the bowl will be approximately 400 ml; this volume will fill the placenta.

e. Repeat steps a-d.

15. Press "spin", "sup out", and "agitate/wash".

16. Close the clamp on the 1.6% NaCl and set the machine to valve 3. Open the reusable adjustable clamp on the 0.9% NaCl-0.2 gm% glucose solution and allow the first of 2 volumes of 0.9% NaCl-0.2 gm% glucose solution to enter the agitating bowl. The volume of 0.9% NaCl-0.2 gm% glucose solution to enter the bowl will be approximately 400 ml; this volume will fill the placenta. Press "spin", "sup out" and "agitate/wash".

a. Repeat step 16.

NOTE: Before continuing, make sure the machine is in the "agitate/wash" mode.

17. Open the clamp on the 0.9% NaCl-0.2% glucose solution and allow the remaining 100 ml to enter the bowl. Press "stop".

18. Place and seal two hand sealer clips 1 inch above the rotating seal and one hand sealer clip 1 inch below the rotating seal.

19. Cut the tubing between the two hand sealer clips located above the rotating seal.

20. Remove the cell processing bag from the centrifuge.

21. Storage of deglycerolized red blood cells:

a. Insert the coupler of the 600 ml transfer pack (Fenwal 4R2021) into the port of the cell wash bowl and transfer the red cells into a 600 ml transfer pack. Using the Sebra heat sealer, seal the tubing 3 times between the bowl and the transfer pack. Cut through the middle heat seal and detach the unit of red blood cells.

b. Label the unit with the word "**Baboon**", the baboon identification number and date washed.

c. If storing the unit prior to transfusion, place the unit into a 4 C refrigerator identified specifically for storage of animal red blood cells.

d. Prior to transfusion, centrifuge the deglycerolized red blood cells at 2982 X g (3400 rpm) for 4 minutes in an RC3B centrifuge at 22 $\pm$ 2 C.

e. Place the centrifuged unit into a plasma expressor. Aseptically insert the coupler of a 600 ml transfer pack (Fenwal 4R2021) and transfer all the visible supernatant. Heat seal the tubing and detach the transfer pack containing the supernatant solution. The red cells will have a hematocrit value of approximately 80-85 V%.

## TABLE 1

## SPEED AND LENGTH OF TIME FOR PROCESSING BLOOD COMPONENTS

1. Fresh whole blood spun at 1615 x g for 4 minutes at 22 C to prepare platelet-rich-plasma (PRP) and a red blood cell concentrate.

2. Non-rejuvenated glycerolized red cells spun at 1248 X g for 10 minutes to prepare a glycerolized red blood cell concentrate.

3. Deglycerolized red cells resuspended in NaCl-glucose solution spun at 2982 x g for 4 minutes at 22 C to remove supernatant hemolysis and prepare a red cell concentrate prior to transfusion.

Where; $RCF = (28.38)(R)\left(\frac{RPM}{1000}\right)^2$

RCF = RELATIVE CENTRIFUGAL FORCE (X g)

R = RADIUS IN INCHES

RPM = REVOLUTIONS PER MINUTE

| RADIUS | ROTOR |
|---|---|
| R = | 9.09 Inches for the HG-4L OR H4000 4-Bucket rotor used in the Sorvall RC-3B or RC-3C centrifuges. |
| R = | 10.25 inches for the H6000A 6-bucket rotor used in the Sorvall RC-3B or the RC-3C centrifuges. |
| R = | 8.90 inches for the JS5.2 4-bucket rotor used in the Beckman J6-B centrifuge. |
| R = | 10.05 inches for the JS4.2 6-bucket rotor used in the Beckman J6-B centrifuge. |

TABLE 2

## METHOD OF ADDITION OF 6.2M GLYCEROL TO NON-REJUVENATED AND REJUVENATED RED BLOOD CELLS

### MODIFIED SOP TO INCLUDE UNITS WITH NET WEIGHTS LESS THAN 150 GRAMS

| GROSS WEIGHT OF UNIT (GRAMS) | NET WEIGHT OF UNIT (GRAMS)* | INITIAL ADDITION OF GLYCEROL (ML) | SECOND ADDITION OF GLYCEROL (ML) | THIRD ADDITION OF GLYCEROL (ML) | TOTAL GLYCEROL ADDED (ML) |
|---|---|---|---|---|---|
| 162 – 192 | 90 – 120 | 25 | 25 | 150 | 200 |
| 193 – 222 | 121 – 150 | 25 | 25 | 200 | 250 |
| 223 – 272 | 151 – 200 | 50 | 50 | 250 | 350 |
| 273 – 312 | 201 – 240 | 50 | 50 | 350 | 450 |
| 313 – 402 | 241 – 330 | 50 | 50 | 400 | 500 |

* Weight of the empty 800 ml primary plastic bag with the integrally attached transfer pack and adaptor port is 72 grams (average)

**TABLE 3**

**NOMOGRAM FOR 12% NACL ADDITION**

| Packed Cell Weight (g) | Volume of 12% NaCl (ml) |
|---|---|
| 90-120 | 20 |
| 121-160 | 30 |
| 161-220 | 40 |
| 221-330 | 50 |

*NOTE: These volumes are calculated assuming a post-thaw hematocrit value of 55 V%. If the hematocrit value is greater than 55 V%, calculate the volume of 12% NaCl to add as follows:*

a. Weigh the unit; determine net weight
b. Measure the hematocrit of the unit using the microhematocrit method
c. Determine the density using the following formula:
    1.1 + {(hematocrit - 20)/1000}
d. Divide the net weight of the unit by the density to achieve the volume of blood in the unit
e. Multiply the volume by 1 - hematocrit (as a decimal) to determine the supernatant volume
f. Divide the supernatant volume by 3 to determine the volume of 12% NaCl to add to the thawed glycerolized red blood cells.